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SCIENTIFIC NOTE

ANOPHELES KOCHI IN IRIAN JAYA DETECTED BY SIZE POLYMORPHISM OF POLYMERASE CHAIN REACTION-AMPLIFIED INTERNAL TRANSCRIBED SPACER UNIT 2

DESMOND FOLEY,[1] PETER EBSWORTH,[2] BAMBANG RISTYANTO[2] AND JOAN HELEN BRYAN[1]

ABSTRACT. *Anopheles kochi* is reported for the 1st time from New Guinea, probably introduced by aircraft. This Oriental species was originally detected by analysis of polymerase chain reaction-amplified rDNA internal transcribed spacer unit 2 (ITS-2). Identification was confirmed by morphologic examination. Size of ITS-2 is presented for 32 species of Australasian and Oriental anophelines to assist morphologic identifications for distribution and vector studies.

KEY WORDS mosquitoes, *Anopheles kochi, Anopheles,* New Guinea, Irian Jaya, rDNA polymerase chain reaction, internal transcribed spacer unit 2, identification

The Australasian Farauti Complex within the genus *Anopheles* contains 7 species (Bryan 1973; Mahon and Miethke 1982; Foley et al. 1993, 1994), some of which are primary vectors of malaria and bancroftian filariasis (Lee et al. 1987). Isomorphic species can be identified using restriction fragment length polymorphism analysis of polymerase chain reaction (PCR)-amplified rDNA fragments containing the internal transcribed spacer unit 2 (ITS-2) (Beebe and Saul 1995). We use this method routinely to identify specimens from New Guinea, the Solomon Islands, and Australia. The DNA was obtained from a single leg and the remainder of the specimen is retained. Recently, in the 1st stage of this identification process, an ITS-2 product with an anomalous size (i.e., 500 base pairs [bp] compared to 700–800 bp, which is normal for the Punctulatus Group) appeared for 1 specimen included in a sample of anophelines from Irian Jaya. Using the morphologic key in Lee et al. (1987) the remainder of the specimen was identified as *Anopheles kochi* Dönitz, a morphologically very distinct species. The size of the PCR product also corresponded to that previously found for *An. kochi* (Table 1).

The collection of this specimen extends the known distribution of this species, which previously has been recorded from most of the Oriental region including Indonesia from Sumatra to the Celebes and the Moluccan Islands of Ternate, Halmaheira, Ceram, Sula, Buru, and Ambon (Lee et al. 1987). The male specimen was captured in a light trap at Kampung Menado (04°33′S, 136°53′E) within 2.5 km of the unloading area at Timika Airport, Irian Jaya, Indonesia, which re-

Table 1. Approximate fragment sizes of polymerase chain reaction-amplified internal transcribed spacer unit 2 from 32 Australasian and Oriental anophelines run on a 2% agarose gel.

Species	Size (base pairs)
Anopheles amictus Edwards	600
An. annularis Van der Wulp	470
An. annulipes A	550
An. annulipes B	550
An. annulipes D	550
An. annulipes G	550
An. balabacensis	800
An. bancroftii	380
An. farauti s.s.[1]	700
An. farauti no. 2[1]	700
An. farauti no. 3[1]	700
An. farauti no. 4[1]	800
An. farauti no. 5[1]	700
An. farauti no. 6[1]	700
An. filipinae Manalang	490
An. flavirostris (Ludlow)	490
An. hilli	800
An. karwari (James)	500
An. kochi	500
An. koliensis[2] Owen	750
An. longirostris Brug	550
An. lungae Belkin and Schlosser	690
An. maculatus s.l.	450
An. mangyanus (Banks)	490
An. meraukensis Venhuis	600
An. novaguinensis Venhuis	700
An. punctulatus[2] Dönitz	700
An. solomonis Belkin, Knight and Rozeboom	690
An. sp. near *punctulatus*[2]	700
An. subpictus s.l.	600
An. tessellatus Theobald	500
Bironella hollandi Taylor	580

[1] These species belong to the Farauti Complex within the Punctulatus Group.

[2] These species belong to the Punctulatus Group.

[1] Tropical Health Program and the Department of Zoology and Entomology, University of Queensland, Brisbane 4072, Australia.

[2] Freeport Public Health and Malaria Control Department, Timika Indah, Timika 98663, Irian Jaya, Indonesia.

ceives flights from Jakarta and Bali. An introduction by aircraft is the most likely explanation for this new geographic record. Extensive surveys failed to find additional specimens, suggesting that *An. kochi* has not established in the area.

This species can be infected experimentally with *Plasmodium falciparum* Celli and Marchiafava and *P. vivax* Golgi (Somboon et al. 1994) and specimens with natural infections have been recorded in Indonesia (Reid 1968). However, the species is not an important vector because of its strong zoophily. Although this introduction does not have serious implications for human health, it demonstrates the need for vigilance at airports.

Fragment sizes for PCR-amplified ITS-2 for 32 Australasian and Oriental anophelines are given in Table 1. Collection details for samples from which these specimens were selected are reported in Foley et al. (1998). Primers and PCR cycling conditions were those of Beebe and Saul (1995). The ITS-2 product was loaded into 2% agarose gels (1% NuSieve and 1% Seakem [FMC Bioproducts, Rockland, ME]) and run for 30 min at 100 mV. *Anopheles farauti* Laveran s.s. and 1-kb ladder were used as internal controls. Sizes range from approximately 800 bp for *An. farauti* no. 4, *An. hilli* Woodhill and Lee, and *An. balabacensis* Baisas to 380 bp for *An. bancroftii* Giles.

Restriction fragment length polymorphism–polymerase chain reaction is sensitive for identification of species within the Punctulatus Group and the inclusion of a foreign species was readily detectable during the 1st stage of this technique. Thus, these fragment sizes are a useful adjunct to morphology to help identify anophelines for distribution and vector studies in the Australasia and Oriental regions.

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